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(54) **Title:** AUTISM GENE

(57) **Abstract:** The present invention concerns genes containing mutations associated with autism its onset and development and also to the encoded proteins of said genes associated with autism, its onset and development and the use of said genes, proteins or protein isoforms. The invention thus also relates to methods of screening for, diagnosis and treatment of autism in human subjects e.g., clinical screening, diagnosis, prognosis, therapy and prophylaxis, as will as for drug screening and drug development.

AMENDED CLAIMS

[Received by the International Bureau on 26 APR 2004 (26.04.04) ; original claims 58 – 61, renumbered]

Claims

1) A method of testing or screening an animal thought to have or to be predisposed to have a neural system disorder comprising detecting the presence of mutation in the NBEA gene or its associated promotor.

2) The method of claim 1, said method comprising: (A) providing chromosomal material from said animal; (B) detecting a modification of the NBEA gene or its promotor in the chromosomal material, wherein said modification is selected from a) substitution, b) deletion, c) frame-shift, d) insertion or e) altered epigenetic control; that causes a loss of biological function in the NBEA gene; and (C) correlating the mutation of said gene with a potential for a neural system disorder.

3) The method of claim 1 or 2, wherein said mutation in the NBEA gene or its promotor is detected by hybridisation with a labelled probe.

4) The method of claim 1 or 2, wherein detection of the presence of the mutation in the NBEA gene is achieved by detecting altered levels of the mRNA transcripts or mRNA precursor.

5) The method of claim 1 or 2, wherein detection of the presence of the mutation in the NBEA gene is achieved by detecting altered levels of the mRNA transcripts or mRNA precursor.

6) A method of claim 1 or 2, wherein said mutation is detected by (A) amplification of the chromosomal material using PCR; (B) sequencing said material to detect the modification of the nucleotide sequence; and (C) correlating the modification of said gene with a potential for neural system disorders.

7) The method of claim 1, wherein, said method comprises: (A) providing biological

material from said animal; (B) detecting the absence, inappropriate, or modified expression of NBEA gene product using labelled ligands to said gene product; and (C) correlating said absence, inappropriate, or modified expression with a potential for neural system disorders.

8) The method of claim 7, wherein the said ligands are polyclonal antibodies.

9) The method of claim 7, wherein the said ligands monoclonal antibodies.

10) The method of any of the claims 1 to 9, characterised in that neural system disorder is autism.

11) The method of any of the claims 1 to 9, characterised in that neural system disorder is associated with any or several symptoms consisting of the group disturbed cognitive functions, disturbed emotional control, disturbed in motor control.

12) The method of any of the claims 1 to 9, characterised in that neural system disorder results from decreased number of Purkinje cells.

13) The method of any of the claims 1 to 9, characterised in that neural system disorder results from brain anomalies.

14) The method of any of the claims 1 to 9, characterised in that neural system disorder results from abnormalities in the cerebellum.

15) The method of any of the claims 1 to 10, characterised in that neural system disorder results from a disturbed the glutamate neurotransmitter system .

16) The method of any of the claims 1 to 10, characterised in that neural system disorder results from reduced levels of the anti-apoptotic protein bcl2 .

17) The method of any of the claims 1 to 16 wherein the animal is a mammal.

18) The method of any of the claims 1 to 16 wherein the animal is a human.

19) Use of a polynucleotide sequence which is hybridisable with a variant NBEA gene, having a deletion, insertion or base substitution which affects transcription and/or translation of the NBEA gene for the manufacture of a polynucleotide probe suitable for diagnosis of a neural system disorder according to method of claim 1.

20) The use of the polynucleotide sequence of the claims 19, wherein the neural system disorders is autism.

21) Use of a polynucleotide fragment comprising the NBEA gene, an allelic variant, minigene or an homologue thereof encoding NBEA or an homologue thereof in the manufacture of a medicament for preventing or treating a neural system disorder

22) Use of a polynucleotide fragment according to claim 21, wherein the medicament is used to treat mammals

23) Use of a polynucleotide fragment according to claim 21, wherein the medicament is used to treat humans.

24) Use of a polynucleotide fragment according to any preceding claims 21, 22 and 23 wherein the neural system disorder is autism.

25) Use of a polynucleotide fragment according to any preceding claims 21, 22 and 23, wherein the neural system disorder is associated with any or several symptoms consisting of the group of disturbed cognitive functions, disturbed emotional control, disturbed in motor control.

26) Use of a polynucleotide fragment according to any preceding claims 21, 22 and 23, wherein the neural system disorder is associated with any or several disorders consisting of the group of decreased number of Purkinje cells, brain anomalies, abnormalities in the cerebellum, disturbed the glutamate neurotransmitter system and reduced levels of the anti-apoptotic protein bcl2.

27) Use of a polypeptide comprising NBEA or a fragment thereof in the manufacture of a medicament for treating a neuronal system disorder

28) Use of a polypeptide according to claim 27, wherein the medicament is used to treat mammals.

29) Use of a polypeptide according to claim 27, wherein the medicament is used to treat humans.

30) Use of a polypeptide according to any of claims 27 to 29, wherein a neural system disorder is autism

31) Use of a polypeptide according to any of claims 27 to 29, wherein a neural system disorder is associated with any or several symptoms consisting of the group of disturbed cognitive functions, disturbed emotional control, disturbed in motor control.

32) Use of a polypeptide according to any of claims 27 to 29, wherein a neural system disorder is associated with any or several disorders consisting of the group of decreased number of Purkinje cells, brain anomalies, abnormalities in the cerebellum, disturbed the glutamate neurotransmitter system and reduced levels of the anti-apoptotic protein bcl2.

33) A polynucleotide comprising a nucleotide sequence, wherein said sequence includes at least one mutation of the NBEA gene, wherein said mutation is selected from a) substitution, b) deletion, c) frame-shift, d) insertion, or e) site-directed mutagenesis that causes a loss of biological function in the NBEA gene.

34) A cell containing the polynucleotide of claim 33.

35) The cell of claim 34 , wherein said cell is a neural cell.

36) The neural cell of claim 35, wherein the cell is derived from an immortal cell line, such as embryonic stem cells, neuronal cell line, or tumour derived cell line.

37) The neural cell of claim 35 and 36, wherein the NBEA gene is under control of a neural-specific promoter or inducible promoters.

38) The neural cell of claim 35, wherein the neural cell is from a wild-type animal.

39) The neural cell of claim 35, wherein the NBEA gene is altered through a naturally occurring mutation.

40) The neural cell of claim 35, wherein the NBEA gene is modified, wherein said mutation is selected from a) substitution, b) deletion, c) frame-shift, d) insertion, or e) site-directed mutagenesis that causes a loss of biological function in the NBEA gene.

41) A non-human animal containing in its genome the polynucleotide of claim 33.

42) A vector containing the polynucleotide of claim 33.

43) An engineered cell comprising a vector comprising the vector of claim 42.

44) A method of screening for a therapeutic agents suitable to treat autism comprising: (A) providing a cell containing a polynucleotide of claim 33; (B) introducing to the cell a agent to be screened; and (C) correlating change in said cell with the activity of the agent.

45) The method of claim 44, wherein changes in said cell are survival, proliferation and differentiation.

46) The method of claim 44, wherein changes in said cell are changes in membrane traffic.

47) The method of claim 44, wherein changes in said cell are changes in vesicular transport.

48) A method of screening for therapeutic agents comprising: (A) providing the non human animal of claim 41 (B) introducing to the animal a agent to be screened; and (C) correlating a change in the development of autism.

49) Use of therapeutic agents obtained by the method according to any of the claims 44 to 48 for restoring normal neurobeachin activity in human neural cells afflicted by a neural system disorder.

50) A method of making a cell with absent, inappropriate or modified NBEA expression comprising: (A) providing a cell; (B) modifying a NBEA gene or the promoter of the NBEA gene in the neural cell, wherein said modification is selected from a) substitution, b) deletion, c) frame-shift, d) insertion and e) post-transcriptional gene silencing by RNA interference that causes a decrease or loss of biological function in the gene; and (C) selecting modified cells.

51) An engineered cell comprising a vector encoding RNAi specific for NBEA mRNA encoded by a heterologues gene relative to the genome of said cell.

52) A method of screening for a therapeutic agents suitable to treat autism comprising: (A) providing a cell of claim 51; (B) introducing to the cell a agent to be screened; and (C) correlating change in said cell with the activity of the agent.

53) The method of claim 52, wherein changes in said cell are survival, proliferation, differentiation or outgrowth.

54) The method of claim 52, wherein changes in said cell are changes the type II protein kinase A phosphorylation pathway.

55) The method of claim 52, wherein changes in said cell are changes in intracellular vesicular transport and/or in membrane traffic

56) An animal with locoregional neural transgenes, wherein said animal comprises a vector encoding RNAi specific for NBEA mRNA encoded by a heterologues gene relative to the genome of said cell

57) A method of screening for therapeutic agents comprising: (A) providing the non human animal of claim 56 (B) introducing to the animal a agent to be screened; and (C) correlating a change in the development of autism.

58) Use of therapeutic agents obtained by the method according to any of the claims 44 to 48 and 52 to 57 to normalise NBEA signal transduction or to normalise the cellular pathway in which NBEA functions in human neural cells afflicted by a neural system disorder.

59) Use of therapeutic agents obtained by the method according to any of the claims 44 to 48 and 52 to 57 for normalising disturbed PKA phosphorylation cascades, intracellular vesicle transport and/or membrane dynamics in human neural cells afflicted by autism.

60) Use of therapeutic agents obtained by the method according to any of the claims 44 to 48 and 52 to 57 for treating autism.

61) A method of screening for a therapeutic agents suitable to treat autism comprising: (A) providing an engineered yeast cell, comprising an introduced nucleotide sequence comprising NBEA gene or an allelic variant, minigene, a synthetic gene or a homologue thereof; (B) introducing to the cell a compound, chemical signal or agent to be screened; and (C) correlating change in said cell with the activity of the compound, chemical signal or agent.

62) The method of claim 61, to screening for compounds, chemical signals or agents

that directly or indirectly affect the biochemistry of NBEA.